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Schou, Kirstine Klitgaard; Weiss Nielsen , Martin ; Jensen, Tim Kåre; Boye, Mette

Publication date:
2014

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Citation (APA):

Schou, K. K., Weiss Nielsen , M., Jensen, T. K., & Boye, M. (2014). *High throughput quantitative PCR to measure prevalence and gene expression of the three major groups of treponemes associated with Digital dermatitis in dairy cows..* Poster session presented at Gordon Research Conferences - Unique Features of Spirochete-Host-Environment Interactions , Ventura, United States.

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High throughput quantitative PCR to measure prevalence and gene expression of the three major groups of treponemes associated with Digital dermatitis in dairy cows

DTU Vet
National Veterinary Institute

Kirstine Klitgaard, Martin W Nielsen, Tim K. Jensen & Mette Boye



Objective

To develop a fast and efficient method for measuring prevalence and gene expression of representatives from the three major groups of *Treponema*, most frequently identified in DD biopsies from cattle.

Introduction

Bovine digital dermatitis (DD), which causes lameness in cattle (Figure 1), is the most serious foot disorder of dairy cows from economic and welfare perspectives. Recent research point towards a polytreponemal etiology of infections, with *Treponema phagedenis*-like, *Treponema medium*/*Treponema vincentii*-like, and *Treponema denticola*/*Treponema pedis*-like phylotypes being highly associated with the disease^{2,3,4}. Still the complex interplay between infecting *Treponema* species and their relative contributions to pathogenesis is largely unknown for this disease and must be elucidated.

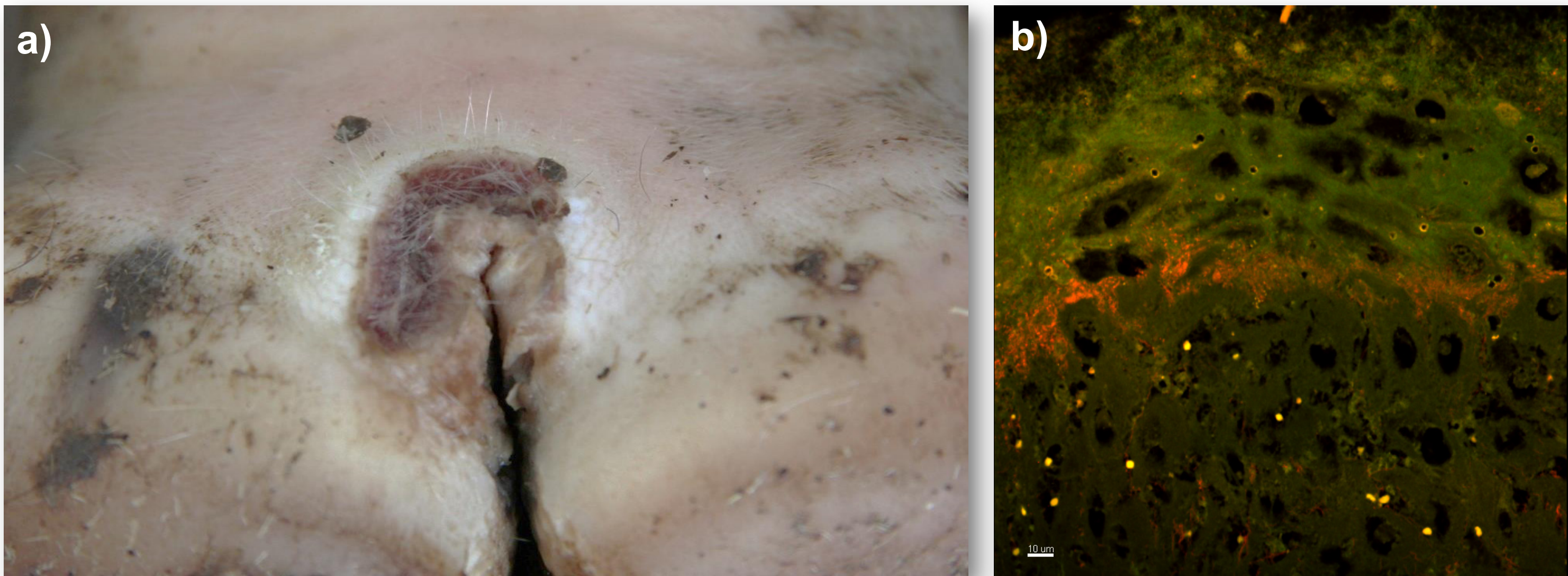


Figure 1. Digital dermatitis lesion (DD12) at a proliferative stage: a) macroscopic, b) microscopic depiction of the same lesion. Bacteria are visualized by Fluorescence *in situ* hybridization applying a *T. phagedenis* specific probe (red) and a general eubacterial probe (green).

| Gene name | Function |
|---------------------------------|--|
| Putative virulence genes | |
| <i>Msp</i> | Major outer sheath protein. Binding to fibronectin, laminin, collagen types I and IV, hyaluronic acid. |
| <i>Hly</i> | Hemolysin. Exhibits hemooxidative and hemolytic activities |
| <i>FlgG</i> | Flagellar basal body rod protein |
| <i>FlgE</i> | Flagellar hook protein |
| <i>hbpB</i> | Hemin-binding protein B Lipoprotein |
| <i>PrtP</i> | Serine protease, dentilisin Possibly involved in bacterial adhesion, tissue penetration and immune evasion |
| <i>fliA</i> | Flagellar filament outer layer protein |
| <i>licCA</i> | Cholinephosphate cytidyllyltransferase |
| <i>mgIA</i> | Galactose/methyl galactoside import ATP-binding protein |
| Reference genes | |
| <i>hexo</i> | Hexokinase family protein (glycolysis) |
| <i>tpiA</i> | Triosephosphate isomerase (Glycolysis) |
| <i>lnt</i> | Apolipoprotein N-acyltransferase (Lipid metabolism) |
| 16S rRNA | Specific for <i>T. pedis</i> , <i>T. phagedenis</i> , <i>T. medium/vincentii</i> and <i>T. denticola</i> |

Table 1. Design of 48.48 Dynamic array.

Methods

From NCBI sequence data (<http://www.ncbi.nlm.nih.gov/>) we designed a high-throughput quantitative PCR (qPCR) assay to differentiate between *T. denticola*-like, *T. medium/vincentii*-like, and *T. phagedenis*-like phylotypes. Samples: cDNA from biopsies taken from cows just after slaughter: Controls (n=3), different stages of DD lesions (n=25). High-throughput reverse transcription qPCR analysis was performed on a 48.48 Dynamic Array (Fluidigm) (Figure 2), targeting 9 putative virulence genes¹ and 3 reference genes (Table 1). 16S rRNA genes were applied for species identification.

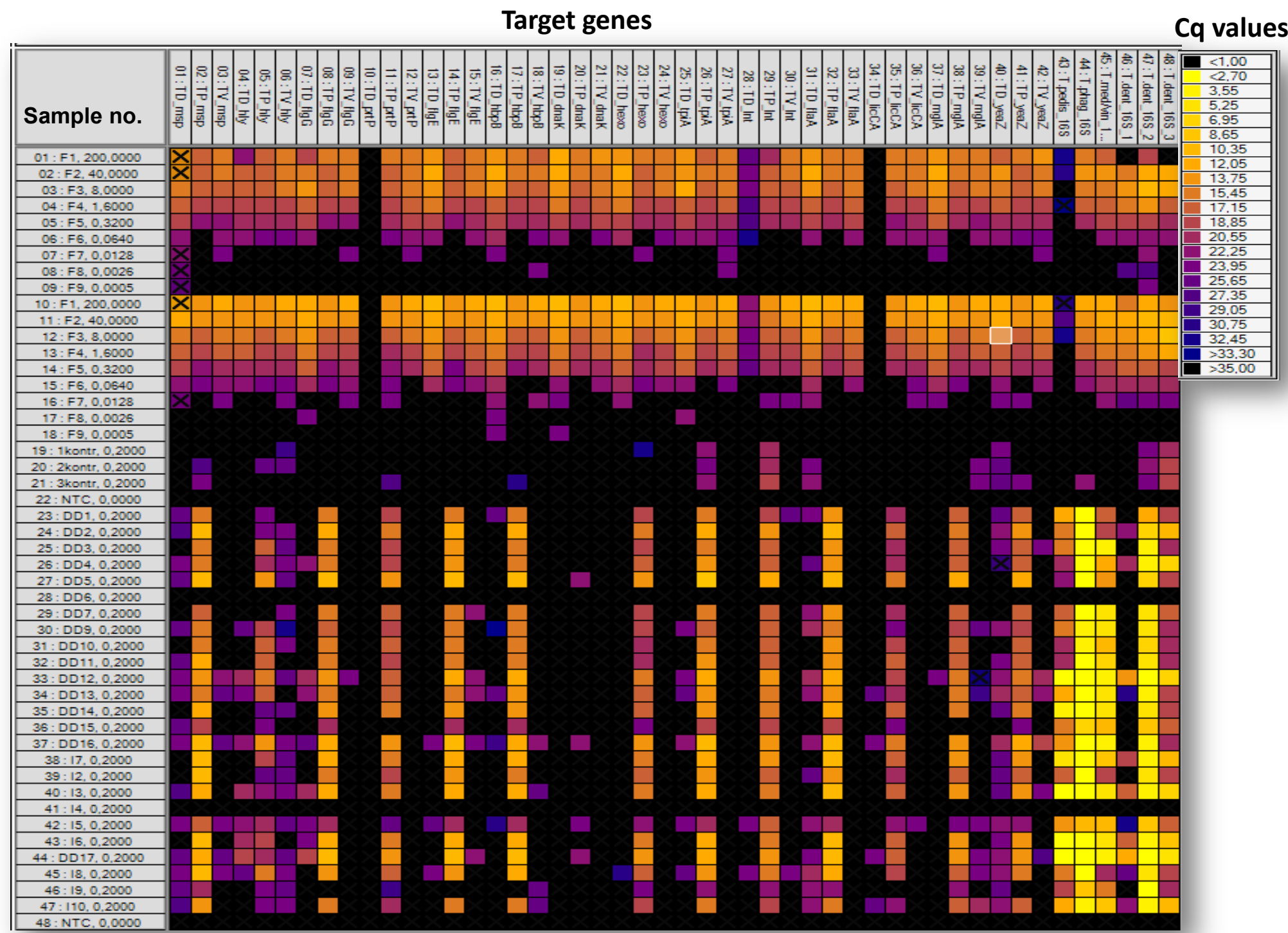


Figure 2. Heat map of expression analysis on 48.48 Dynamic array.

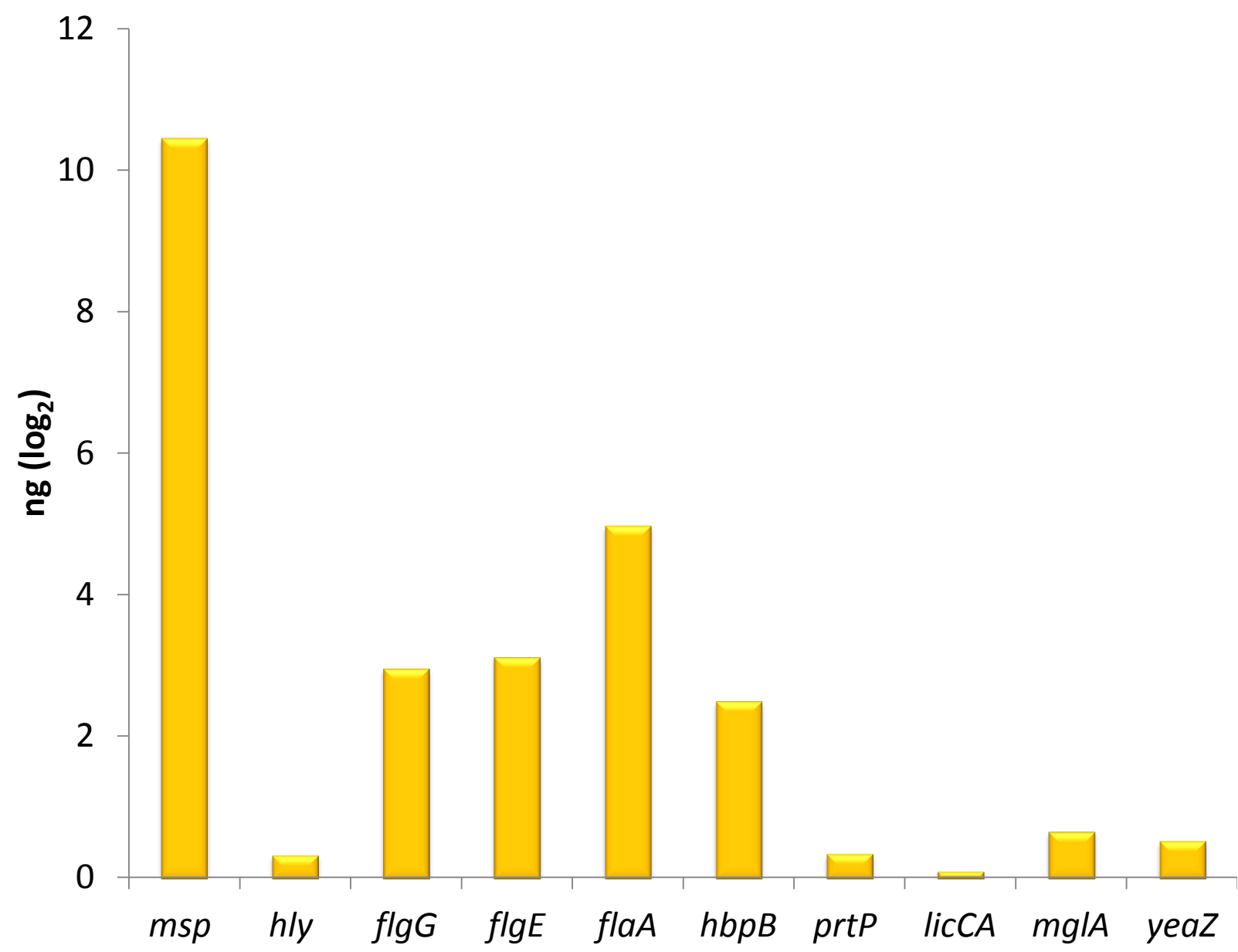


Figure 3. Mean expression (\log_2) of *T. phagedenis*-like phylotypes in positive samples (n=23).

Results

Although all three major groups of DD-related treponemes appeared to be present in most of the lesions (16S rRNA positive results), the expression results indicated that only *T. phagedenis*-like species were metabolically active (Figure 2). *T. denticola*/*T. pedis*-like and *T. medium*/*T. vincentii*-like species may either be present in low numbers and/or they may be less active than *T. phagedenis*. An example of the distribution of *T. phagedenis*-like species in DD lesions is shown in Figure 1b. From this picture it can be seen that *T. phagedenis*-like bacteria are highly active in the deeper part of the lesions, which is in good accordance with the expression results. The most active genes were related to functions such as host adhesion and motility (Figure 3).

Conclusion

The method of parallel gene expression analysis presented here needs further optimization but seems promising and may be broadly applicable for high throughput investigation of the infection dynamics of *Treponema* in DD lesions.

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Acknowledgements: Anastasia Isbrand is acknowledged for her technical support in the laboratory.

Funding: The project is funded by The Danish Council for Independent Research (project no. 12-127537).

